Serum 25- Hydroxy Vitamin D levels in Type 2 Diabetes Mellitus- A Comparative study

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Abstract

Background: Type 2 diabetes mellitus (DM) is a disease of impaired carbohydrate metabolism. Vitamin D deiciency and diabetes mellitus are two common conditions and they are widely prevalent across all ages, races, geographical regions, and socioeconomic conditions. The present study aimed to see the effect of 25 hydroxy (OH) vitamin D levels on glycemic control and to show their role in pathogenesis.

Materials and Methods: It was a case control study. The study included 100 cases of clinically diagnosed type 2 DM and 100 age and sex matched healthy controls. Venous blood sample was analysed for fasting blood sugar(FBS), post prandial blood sugar (PPBS), serum 25-OH vitamin D and glycosylated hemoglobin(HbA1c) in both cases and controls. Statistical analysis was done using student't' test. Pearson's correlation was performed to establish the relationship between study variables.

Results: There was decreased levels of serum 25-OH vitamin D levels (p<0.001) in cases as compared to the controls. There was negative correlation between 25-OH vitamin D levels and glycosylated hemoglobin (HbA₁c) in cases of type 2 DM.

Conclusion: 25-OH Vitamin D levels were lower in patients of type 2 DM as compared to control group. Early detection of vitamin D deficiency and supplementation may help in improvement of glycemic control and prevent complications.

Key words: Type 2 DM, 25 OH Vitamin D, glycosylated hemoglobin



Introduction

Type II Diabetes Mellitus (DM) is a metabolic disorder primarily characterized by hyperglycemia. It is caused by insulin resistance and/or relative insulin deficiency¹. It is a chronic condition with serious morbidity, increased mortality and is rapidly becoming a global pandemic. The growing incidence and prevalence of diabetes has made to go for innovative approaches in management of the disease. Type 2 diabetes mellitus (T2DM) is a predominant public health concern worldwide, accounting for 90% of the cases of diabetes globally^{2,3,4}.

Vitamin D also known as sunshine vitamin, a fat soluble vitamin has diverse biological effects.Vitamin D3 is synthesized from 7-dehydrocholesterol in the skin. The vitamin D binding protein transports the vitamin D3 to the liver where it undergoes hydroxylation to 25(OH)D (the inactive form of vitamin D) and then to the kidneys where it is hydroxylated by the enzyme 1 alpha hydroxylase to 1,25(OH)D, its active form. This enzyme is also present in a variety of extrarenal sites, including osteoclasts, skin, colon, brain, and macrophages, which may be the cause of its broad-ranging effects⁵.

Vitamin D deficiency has been a global pandemic for a while^{6,7}, yet the level of attention given by the scientific and clinical community was only recently stimulated, primarily because of the pleiotropic effects of this hormone outside the skeletal system. Vitamin D deficiency has been consistently associated with hypertension, diabetes mellitus, cardiovascular disease, stroke, multiple sclerosis, inflammatory bowel disease. It has been shown to be related with a lower risk for development of DM in high risk patients (Pittas et al. 2012). It has been also described in metabolic syndrome (Kayaniyil et al.2013).

Studies on this parameter have been done in other areas but very few in our locality. Hence, we conducted this study with following aims:

- 1. To estimate serum 25-hydroxy vitamin D levels in cases of type 2 DM and to compare it with those of healthy controls.
- 2. To correlate serum 25-hydroxy vitamin D levels with HbA1c in patients with type 2 DM.

Material and Methods

Study participants: This was a case control study. The study was carried out on 100 cases of clinically diagnosed type 2 diabetes mellitus in the age group 30-70 years attending the Medicine OPD at SNMC and HSK hospital, Navanagar, Bagalkot. Hundred (100) age and sex matched healthy subjects were taken as controls. Study duration was from January 2014 to august 2014. Ethical clearance was obtained from the

institute's ethical clearance committee. Informed consent was taken from the cases and controls after explaining the procedure. Diabetes Mellitus was diagnosed as per the WHO diagnostic criteria⁸. Vitamin D deficiency was defined as serum values < 20 ng/ml⁹ (Normal->30ng/ml, Insufficiency-20-29.9ng/ml, Deficiency<20ng/ml).

Exclusion criteria: Patients of DM with other microvascular complications, individuals with severe inflammatory diseases, infections, cardiac, hepatic or renal diseases were excluded from the study. Patients on diuretics, individuals taking drugs that affect blood glucose levels, pregnant and lactating women were also excluded

Biochemical analysis: A sample of 3 ml venous blood was collected in both fasting and post prandial state under aseptic precautions. It was allowed to clot and serum was separated by centrifugation.

The following parameters were studied.

- FBS and PPBS –Glucose oxidase peroxidase method^{10,11}. (kits supplied by Erba Diagnostics). The parameters were read using semi auto analyser (STAT FAX 3300).
- 2. HbA1c was estimated by Nycocard reader II¹².
- 3. 25-OH Vitamin D levels were measured in serum by chemiluminescence immunoassay (CLIA) method using Snibe Maglumi 1000.

If electing to test vitamin D status, serum 25 hydroxy vitamin D is the accepted biomarker¹³. Although1, 25-OH-D is the active circulating form of

vitamin D, measuring this level is not helpful because it is quickly and tightly regulated by the kidney.

Statistical Methodology: Data was expressed in terms of mean \pm SD. Chi- square test was applied to estimate the difference between the two groups of population. Unpaired 't'-test was used to study the changes in serum 25 OH vitamin D levels between the study groups. Pearson correlation was performed to establish the relationship between study variables. p value <0.05 was considered statistically significant.

Results

This was a comparative case control study conducted on 100 cases of type 2 DM (n=100) and 100 age and sex matched healthy controls (n=100). Serum 25-OH Vitamin D was estimated, analysed and correlated with HbA1c, FBS and PPBS. The results were expressed as mean±standard deviation.

The mean age (in years) of cases was 46.5 ± 11.0 years and that of controls was 47 ± 11.3 years and was not significant. (p=0.20). **Table 1** shows comparison of serum 25-OH Vitamin D, FBS, PPBS and HbA1c levels in both groups and was statistically significant(p<0.05). The mean serum 25-OH Vitamin D levels (ng/mL) in cases was 13.9 \pm 5.3 ng/mL and that in controls was 30.0 ± 2.17 ng/mL. And was highly significant (p <0.0001).

Serum 25-OH Vitamin D levels and HbA1c: There was significant negative correlation between serum 25-OH Vitamin D levels and HbA1c, r = -0.65, p < 0.001 and was highly significant. (**Table 2**)

Characteristics	Groups	Mean±SD	t	Р
FBS (mg/dL)	Cases	214.5±54.4	10.1	0.001^{*}
	Controls	89.2±11.3		
PPBS(mg/dL)	Cases	270.3±60.6	18.2	0.001^{*}
	Controls	112.9±.6		
HbA1c(%)	Cases	7.8±0.7	14.8	0.001*
	Controls	5.4±0.5		
25-OH Vitamin	Cases	13.9 ± 5.3	16.5	0.001
D (ng/ml)	Controls	30.0±2.17		

 Table 1: Comparison of FBS, PPBS, 25-OH Vitamin D and HbA1c levels in both groups

*statistically highly significant, FBS- Fasting blood sugar, PPBS-Post prandial blood sugar, HbA1c –Glycosylated Hemoglobin

Table 2:	Correlation	between	study	variables

Correlation between	Pearson's Correlation Coefficient(r)	Significance	
FBS and 25-OH	-0.48	p < 0.001	Highly significant negative
Vitamin D			correlation
PPBS and 25-OH	-0.60	p < 0.001	Highly significant negative
Vitamin D		-	correlation
HbA1c and 25-OH	-0.65	p<0.001	Highly significant negative
Vitamin D			correlation

FBS-fasting blood sugar, PPBS-post prandial blood sugar, HbA1c-glycosylated haemoglobin

Discussion

Our study demonstrated decreased levels of vitamin D in cases of type 2 DM. We also found a significant negative correlation between 25-OH vitamin D and glycemic status (HbA1c, FBS, PPBS) in type 2 DM.

There are several lines of evidence supporting the role for vitamin D in pancreatic beta-cell function. Sheena et al¹⁴ examined the cross-sectional association between vitamin D and beta-cell dysfunction in subjects at risk for type 2 diabetes and showed a positive association between vitamin D and beta-cell function. A high prevalence of hypovitaminosis D was noted among women with type 2 diabetes. Hyper responsive insulin secretion after a glucose challenge has been found in older men with hypovitaminosis D¹⁵.

Vitamin D may act in two possible pathways; it may act directly to induce beta-cell insulin secretion by increasing the intracellular calcium concentration via non-selective voltage-dependent calcium channels or it may mediate activation of beta-cell calcium-dependent endopeptidases to produce the cleavage that facilitates the conversion of proinsulin to insulin. In peripheral insulin-target tissues, vitamin D might directly enhance insulin action through stimulation of the expression of insulin receptors and regulation of the calcium pool¹⁶.

Type-2 DM was found to be associated with an increase in the levels of the tumour necrosis factoralpha and beta, the C reactive protein, the plasminogen activator inhibitor-1 (PAI-1), and interleukin-6 (IL-6)¹⁷⁻ ¹⁹. The increase in these inflammatory mediators may precede and even predict the development of type-2 DM. In support of this concept, is the finding that VDR has been found on almost all the cells of the immune system and that vitamin D can repress the type 1 cytokines, inhibit dendritic cell maturation, and upregulate the regulatory T cells. Furthermore, immune cells such as macrophages contain 1ά-hydroxylase that can be upregulated by the inflammatory mediators and not by PTH. Vitamin D also suppresses the antigenpresenting capacity of the macrophages, it modulates the development of the CD4 lymphocytes and it inhibits the production of IFNc (interferon c) and IL-2 (interleukin 2), among other cytokines. These cytokines are known to activate the macrophages and the cytotoxic T cells, which in turn can lead to the destruction of the pancreatic islets. By the modulation of the immune and the inflammatory processes, vitamin D may also decrease insulin resistance and increase the insulin secretion in type-2 DM, which are the two characteristic defects in this condition.

Vitamin D may have a beneficial effect on insulin action either directly, by stimulating the expression of insulin receptors thereby enhancing insulin responsiveness for glucose transport²⁰, or indirectly via its role in regulating extracellular calcium ensuring normal influx in to membrane and adequate intracellular cytosolic calcium. Calcium is essential for insulin-mediated intracellular processes in insulinresponsive tissues such as skeletal muscle and adipose tissue with a very narrow range of intracellular calcium needed for optimal insulin-mediated functions. Changes in calcium levels in primary insulin target tissues may contribute to peripheral insulin resistance via impaired insulin signal transduction leading to decreased GLUT-4 activity²⁰.

The role of vitamin D in type 2 DM is also suggested by a seasonal variation in glycemic control reported in patients with type 2 diabetes being worse in the winter, which may be due to prevalent hypovitaminosis D as a result of reduced sunlight in winter.

From the above discussion, it is clear that vitamin D has a significant role to play in the molecular mechanisms of the synthesis, secretion and the peripheral sensitivity of insulin, pathogenesis of type 2 DM.

Conclusion

We conclude that 25 OH vitamin D is correlated with glycemic status in type 2 DM and has a role in pathogenesis of type 2 DM and its complications. Further early detection of vitamin D deficiency and supplementation may help in improvement of glycemic control and prevent complications of type 2 diabetes mellitus.

Future experimental studies are needed to establish the underlying mechanisms of the present study results.

Limitations of the study

Small number of the sample

Confounders like diet, physical activity were not included

Doses of medications taken by patients was not included. Dose dependent effects couldn't be studied.

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